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Communication

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Simultaneous Determination of Binding Constants for Multiple Carbohydrate Hosts in Complex Mixtures

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Supporting Information

ABSTRACT: We describe a simple method for the simultaneous determination of association constants for a guest binding to seven different hosts in a mixture of more than 20 different oligosaccharides. If the binding parameters are known for one component in the mixture, a single NMR titration suffices to determine binding constants for all other detectable and resolvable hosts. With the use of high-resolution $^{1}H-^{13}C$ HSQC experiments, complexes of amphiphiles with more than 10 different maltooligosaccharides can be resolved. Hereby, the binding capabilities of a set of structurally related hosts can be quantitatively studied to systematically explore noncovalent interactions without the need to isolate each host.

Molecules and molecular interactions in nature exist not in isolation, but within complex chemical networks.[1] Nevertheless, chemists wishing to explore molecular recognition usually conduct their experiments to quantify binding interactions on individual isolated compounds.[2] Systems chemistry—the study of complex mixtures of interacting synthetic molecules—is still in its infancy. Two important prerequisites for the development of this field are the availability of analytical tools to resolve and distinguish individual components in a mixture and the availability of simple methods to simultaneously quantify multiple competing binding interactions in mixtures.

In this communication, we describe a simple NMR spectroscopy method to simultaneously determine association constants for the interaction of a guest with multiple different hosts present in a complex mixture. Improving upon the few previously described examples of multiple binding constant estimation from analysis on mixtures using affinity chromatography,[3] capillary electrophoresis[4] and diffusion-ordered NMR spectroscopy,[5] our method should be generally applicable so long as signals from the different 1:1 host/guest complexes in fast exchange can be resolved in the NMR spectrum. We showcase our method in a systematic study of the binding of $\alpha(1-4)$-linked-glucopyranose oligosaccharides to amphiphiles with aliphatic tails (Figure 1). We highlight how the rapid extraction of binding data from a network of equilibria circumvents the need to isolate individual components in order to study noncovalent interactions in chemical systems.

While the binding properties of cyclodextrins (cyclic $\alpha(1-4)$-linked glucanopyranose oligosaccharides) have been thoroughly studied leading to numerous industrial applications,[6] their linear counterparts, maltooligosaccharides, have received very little attention as molecular hosts. It is known that long maltooligosaccharides can wrap around and bind or solubilize hydrophobic guests, such as carbon nanotubes,[7] molecular wires,[8] lipids and surfactants,[9] in left handed helices. The influence of oligosaccharide length on binding strength and conformation, however, has yet to be clearly defined. Systematic studies of oligosaccharide binding are hampered by analytical limitations—in particular, signal overlap and difficulties distinguishing and resolving homo-oligosaccharides using traditional NMR experiments—and the challenge of isolating specific length

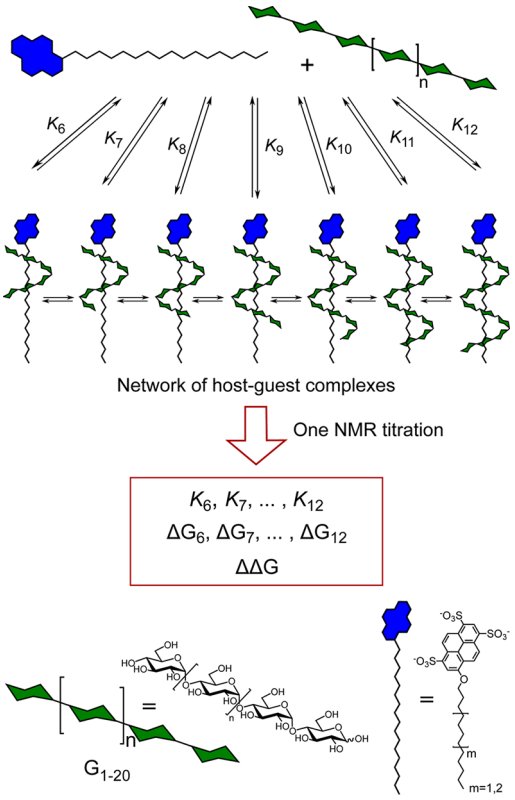

Figure 1. In this study, we examine the interaction of maltooligosaccharides (G_1-G_{20}) with HPTS-C_{16} and HPTS-C_{12} in mixtures and extract binding parameters for each complex from a single titration.

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maltooligosaccharides that are usually produced as mixtures either by enzymatic synthesis or the enzymatic degradation of naturally occurring polysaccharides.

We recently described the use of sensitivity-enhanced $^{1}H-^{13}C$ HSQC spectra with optimized spectral width in the ^{13}C dimension to rapidly resolve, distinguish and quantify different maltooligosaccharides in mixtures via subtle differences in the chemical shifts of their anomeric (hemiacetal) signals.[10] Beyond identification and quantification or carbohydrates, such high-resolution NMR methods provide the analytical infrastructure to enable the study of molecular recognition by multiple hosts present in complex carbohydrate mixtures.

In the course of our recent investigations into the development of molecular probes and sensors for polysaccharides,[11,12] we have examined by means of ^{1}H NMR spectroscopy the interaction between fluorescent amphiphiles based on 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS) and short commercially available maltooligosaccharides (maltohexoase (G_6), maltoheptaose (G_7) and maltooctaose (G_8)) or mixtures of longer maltooligosaccharides (Figure 1). With traditional ^{1}H NMR spectroscopy titrations, the binding of the amphiphiles by long maltooligosaccharides in a mixture was apparent, but we were unable to identify the individual complexes formed or to obtain association constants for these interactions due to congestion of carbohydrate signals in mixtures and overlap with the amphiphile and water signals in the NMR spectra.

Encouraged by the remarkable resolution of maltooligosaccharides achievable using HSQC NMR spectroscopy on complex carbohydrate mixtures, we chose to utilize similar experiments to examine the binding interaction of HPTS-C_{16} with a mixture of maltooligosaccharides ranging from glucose to G_{20} (see Figure S1, for length distribution in the mixture). The maltooligosaccharide mixture (10 mg/mL) was titrated with HPTS-C_{16} (10 mM) in D_2O. The $^{1}H-^{13}C$ HSQC signals for the β-anomeric reducing end protons were monitored (Figure 2). In the absence of any guest, the signals for G_2-G_{20} are completely overlapped. However, upon addition of HPTS-C_{16}, the signals for G_5 and longer shift significantly upfield in the ^{1}H dimension with the signals for G_5-G_{12} becoming distinct from one another. Signals from G_1-G_8 were identified using reference compound mixtures (see Figure S2) and G_9-G_{12} were assigned by extrapolation with reference to their relative concentrations in the mixture. Longer oligosaccharides up to at least G_{20} are present in the mixture at low concentrations (as seen by HPLC analysis of the mixture, see Figure S1) but were not detectable in these experiments. Using 20 min HSQC experiments, G_{12} present at a concentration of approximately 300 μM was just detectable.

Figure 3 shows a plot of the normalized chemical shift changes in the ^{1}H dimension of G_7-G_{12} observed upon titration of the mixture with HPTS-C_{16}. The binding isotherms for the shorter oligosaccharides display a distinctly sigmoidal curvature, which indicates that these oligosacharaides bind more weakly than some of the longer oligosaccharides; when the guest concentration is limited, weaker receptors will be largely unbound as the complexation of stronger binders dominates. This behavior was not unexpected, as longer oligosaccharides have the possibility to form more well-defined binding sites as they assemble into a helix around a hydrophobic guest.

Leito and co-workers recently described an NMR method for the simultaneous determination of relative association constants $\Delta\log K$ for the binding of acetate in mixtures of up to three receptors.[13] They highlighted the relationship between the ratio of association constants for pairs of hosts (K_a and K_b) and the

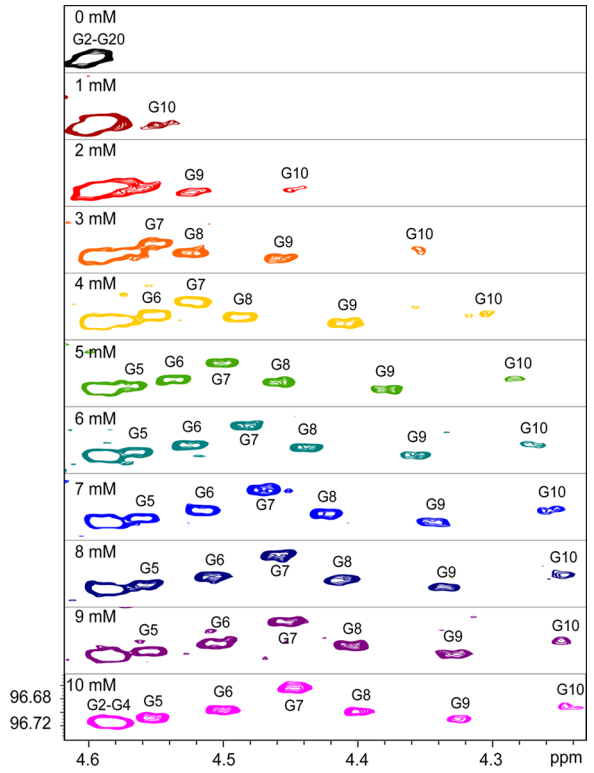

Figure 2. $^{1}H^{13}C$ HSQC spectra (300 K, D_2O) showing the H1β reducing end signals of a mixture of maltooligosaccharides (G_1-G_{20}) in the presence of increasing concentrations of HPTS-C_{16}.

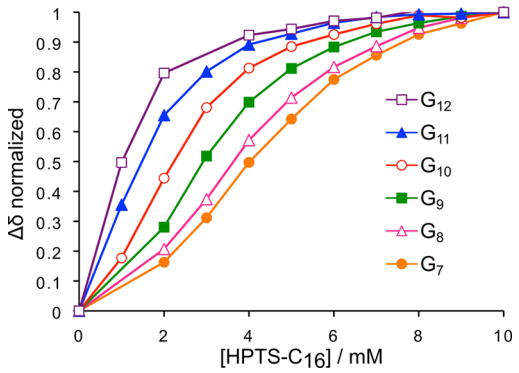

Figure 3. ^{1}H chemical shift changes ($\Delta\delta$) observed for the H1β reducing end signal of maltooligosaccharides in the mixture upon titration with HPTS-C_{16} in D_2O normalized to the chemical shift change observed in the presence of 10 mM HPTS-C_{16}.

degree of association for each host (α_a and α_b). For a system in fast exchange this can be written in terms of the chemical shift change observed upon addition a specific amount of guest for each host ($\Delta\delta_a$ and $\Delta\delta_b$) and the maximum chemical shift change observable for each host upon saturation of the mixture with guest ($\Delta\delta_{a,max}$ and $\Delta\delta_{b,max}$) (eq 1), where $\Delta\delta = \delta - \delta_H$ and $\Delta\delta_{max} = \delta_{HG} - \delta_H$, and δ, δ_H, and δ_{HG} are the observed chemical shift and the chemical shifts of the free and bound hosts, respectively.

$$\frac{K_a}{K_b} = \frac{\alpha_a(1-\alpha_b)}{\alpha_b(1-\alpha_a)} = \frac{\Delta\delta_a(\Delta\delta_{b,max} - \Delta\delta_b)}{\Delta\delta_b(\Delta\delta_{a,max} - \Delta\delta_a)} \quad (1)$$

From eq 1, it can be seen that the ratio of association constants K_a/K_b is independent of the guest concentration and the total host concentration. This means that relative association

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Table 1. Binding parameters for the interaction of maltooligosaccharides with HPTS-C_{16} (in D_2O at 300 K)

guest	K (M^{-1})[b]				$\Delta\delta_{max}$ (ppm)[c]				ΔG (kJ mol^{-1})	
method[a]	a	b	c	d	a	b	c	d	a	average[d]
G5	370				0.08				−14.7	
G6	420		430	400	0.17		0.17	0.17	−15.0	−15.0
G7	470	450		420	0.25	0.26		0.26	−15.3	−15.2
G8	660	700	720		0.29	0.29	0.28		−16.2	−16.4
G9		1500	1400	1300		0.33	0.33	0.34		−18.1
G10		3300	3000	2800		0.39	0.39	0.40		−20.0
G11		6400	6900	6600		0.44	0.44	0.44		−21.9
G12			16000	15000			0.48	0.48		−24.1

[a]Binding parameters were determined using methods a−d as follows: (a) from titration of the isolated oligosaccharide; (b) from titration of the mixture fitted relative to the binding constant determined independently for G_6; (c) for G_7; and (d) for G_8. [b]Estimated fitting errors for G_6-G_{11} < 5%, estimated fitting errors for G_{12} = 11%. [c]Estimated fitting errors <2%. For individual uncertainties see Table S1. [d]Average value calculated from K values determined relative to G_6, G_7 and G_8

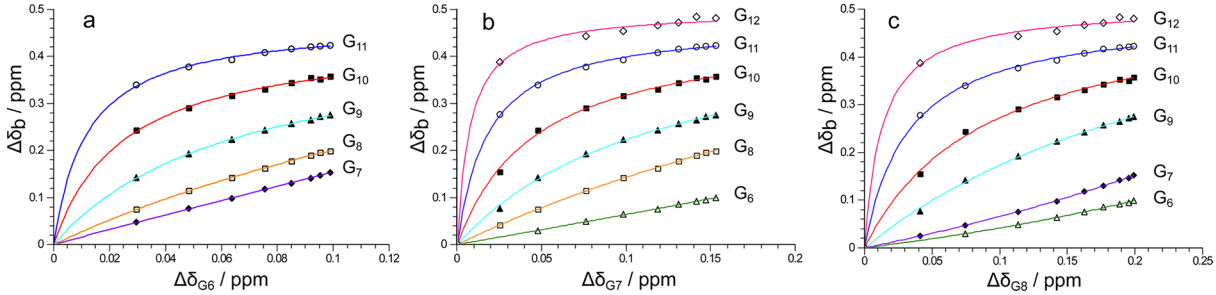

Figure 4. Chemical shift changes ($\Delta\delta_b$) observed for the anomeric H1β signals of maltooligosaccharides G_6-G_{12} in the maltooligosaccharide mixture upon titration with HPTS-C_{16} plotted against the chemical shift changes ($\Delta\delta_a$) for (a) G_6, (b) G_7, and (c) G_8 and fitting of the data to eq 2.

constants can in principle be determined in mixtures where the number and concentration of potential host is unknown.

Using the method described by Leito and co-workers[13] the chemical shifts of the various host/guest complexes, δ_{HG}, must be known in order to be able to estimate the degree of association. While this may be possible to estimate with some accuracy in simple systems with synthetic hosts and strong binding, where a high concentration of guest may be added to saturate the system, it will often be impossible in more complex mixtures of many weak binders or where the guest has limited solubility or availability. We reasoned, however, that if the association constant, K, and the maximum chemical shift change, $\Delta\delta_{max}$, are known for one host (H_a) then it should be possible to perform a titration in which increasing concentrations of guest are added to the host mixture to determine simultaneously both $\Delta\delta_{max}$ and K for all other hosts (H_b). By plotting $\Delta\delta_b$ as a function of $\Delta\delta_a$, and knowing K_a and $\Delta\delta_{a,max}$, the two unknowns, K_b and $\Delta\delta_{b,max}$ can be determined by fitting of the data to eq 2.

$$\Delta\delta_b = \frac{\Delta\delta_{b,\max}\Delta\delta_a K_b}{\Delta\delta_{a,\max}K_a - \Delta\delta_a K_a + \Delta\delta_a K_b} \quad (2)$$

To test this method, we began by determining independently K and $\Delta\delta_{max}$ for G_6, G_7 and G_8 by performing titrations on these maltooligosaccharides in isolation (Table 1, columns 2 and 6, see Figures S3 and S4). The chemical shift changes observed for G_6-G_{12} upon titration of the maltooligosaccharide mixture with HPTS-C_{16} (Figure 2) were plotted against the chemical shift changes observed for G_6, G_7 and G_8 in the same mixture (Figure 4a−c). The data were each fitted to eq 2 to give independent estimates of K and $\Delta\delta_{max}$ for the binding of HPTS-C_{16} with each of G_6-G_{12} (Table 1, columns 3−5 and column 7−9). There was little variation between the values determined, which indicated the robustness of the method yielding a typical precision for K on the order of 5% around the mean and for $\Delta\delta_{max}$ of 3%. Furthermore, there was excellent agreement with the values of $\Delta\delta_{max}$ and K obtained from titrations on the isolated oligosaccharides G_6-G_8 (Table 1, columns 2 and 6).

The possibility to accurately determine multiple binding constants from titrations performed on mixtures enables the rapid quantitative assessment of the binding abilities of potential hosts. In particular, it can facilitate the study of series of related hosts (here, for example, a set of homooligomers) in order to conduct systematic investigations of noncovalent interaction in host−guest systems.[14] As we were interested to study the hydrophobic interaction between α(1−4) glucans and aliphatic chains, we chose to augment our collection of binding data obtained for HPTS-C_{16} with a complementary set of binding energies for the interaction of maltooligosaccharides with an amphiphile with a shorter tail, HPTS-C_{12}. First, maltooctaose was titrated with HPTS-C_{12} to obtain K_{G8} and $\Delta\delta_{G8,max}$ as reference values (see Figures S5 and S6). Then, the maltooligosaccharide mixture (G_1-G_{20}) was titrated with HPTS-C_{12} and the binding parameters for complexation of G_5-G_{12} were determined as described above for HPTS-C_{16} (see Figures S7−S9 and Table S2).

The relationship between the free energy change upon complexation, ΔG, for HPTS-C_{16} and HPTS-C_{12} is plotted against the degree of polymerization of the oligosaccharides analyzed (Figure 5). In both cases, ΔG was a linear function of the number of glucose monomers (G_n) for n = 8−12, with each additional glucose residue stabilizing the complex by 1.95 kJ mol^{-1} for HPTS-C_{16} and 1.55 kJ mol^{-1} for HPTS-C_{12}. It has previously been reported on the basis of powder diffraction X-ray analysis and CD spectroscopy that long α(1−4) glucans bind

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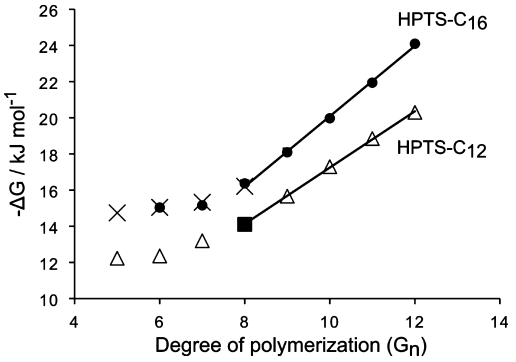

Figure 5. Plot of the binding free energy ($-\Delta G$) versus the degree of polymerization of the maltooligosaccharide (G_n) for complexation of HPTS-C_{16} and HPTS-C_{12}. Filled circles, fitted data for HPTS-C_{16}; crosses, individually determined values for HPTS-C_{16}; open triangles:, fitted data for HPTS-C_{12}; filled square, individually determined data for HPTS-C_{12} and G_8. For HPTS-C_{16}, n = 8−12, $\Delta\Delta G$ = 1.95 kJ mol^{-1} glucose^{-1}; for HPTS-C_{12}, n = 8−12, $\Delta\Delta G$ = 1.55 kJ mol^{-1} glucose^{-1}.

aliphatic guests in helices with six glucose residues per turn.[9,15] The current results suggest that 7 glucose residues are required to assemble a stable helix around the guest. Each additional glucose residue then has an equal stabilizing effect on binding. Once organized into a helix, there is likely a minimal entropic penalty associated with each additional glucose. Instead there is a linear increase in stabilization energy most likely due to the incremental addition of intramolecular hydrogen bonding between parallel glucose in the helix (i.e., between G_i and G_{i+6}) and a systematic increase in the hydrophobic surface presented toward the interior binding cavity. HPTS-C_{16} binds significantly more strongly to the oligosaccharides than HPTS-C_{12} and $\Delta\Delta G$ is larger for HPTS-C_{16} than for HPTS-C_{12}. This can be explained by the fact that for the longer amphiphile there is an increased chance of binding the oligosaccharide as it has a larger available hydrophobic surface area; each additional glucose provides an even stronger interaction with this surface. It is probable that there is a limit to the oligosaccharide length beyond which ΔG is no longer linearly correlated with n because the oligosaccharide is sufficiently long to completely wrap around the aliphatic tail; this limiting length would likely be longer for HPTS-C_{16} compared with HPTS-C_{12}.

In conclusion, we have described a method for the determination of multiple binding constants in complex mixtures. If the binding constant, K, and the chemical shift of one host/guest complex, δ_{HG}, in the mixture are known, the binding constants for all other detectable and resolvable complexes may be determined from a single NMR titration. This is the case even when the concentrations of the various hosts are unknown, where there may be impurities and/or undetectable competing hosts present in the mixture, and when δ_{HG} is not known for the other hosts. We highlight the power of high-resolution NMR experiments to study binding phenomena in complex chemical networks of homo-oligosaccharides. The rapid acquisition of numerous binding parameters from few experiments facilitates the systematic studies of binding by sets of related compounds. We anticipate that the application of this methodology to extract binding constants from mixtures should have wide ranging applications for the study of chemical systems, both synthetic and naturally occurring, for which the isolation and individual study of each component is undesirable, or not feasible.

■ ASSOCIATED CONTENT

Supporting Information

Additional NMR spectra, fitted binding isotherms, error estimations and derivation of equations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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